REMARKS

Claims 1-7, 9, 11-15, 17, 19, 21-30, 34, 36, 38-44, 49-62, and 75-77 are pending. Claims 1-3, 6, 9, 15, 23-25, and 27-30 have been amended. Claims 4, 5, 7, 14, 17, 26, 36, 39-42, 44, 49, and 54-62 are original. Claims 11-13, 19, 21, 22, 34, 38, 43, 50-53, and 75-77 have been previously presented. Claims 8, 10, 16, 18, 20, 31-33, 35, 37, 45-48, and 63-74 have been canceled. No new matter has been introduced by the amendment.

1. Summary of The Present Invention As Illustrated by Below Embodiments

A significant problem involving current crystallization approaches is determining the conditions for forming crystals with optimal diffractive properties. Screening for optimal conditions typically involves a number of parameters, for example, types and concentrations of precipitation agent, buffers, salts, and other chemical additives (e.g., metal ions, salts, small molecular chemical additives, cryoprotectants, etc.). The claimed methods allow higher throughput automated systems with improved speed, sample economy, and entirely new methods of controlling crystallization.

The presently claimed methods allow one to rapidly conduct a huge number of crystallization experiments – using very little sample. One application of these devices and methods is to provide novel and efficient means for high-throughput crystallization of soluble and membrane proteins.

For example, as shown in Fig. 5, which is duplicated below, multiple plug-fluids (containing e.g., precipitating agent, compound to be precipitated, solvent, etc.) can be introduced into one or more inlet ports 501 to 507.

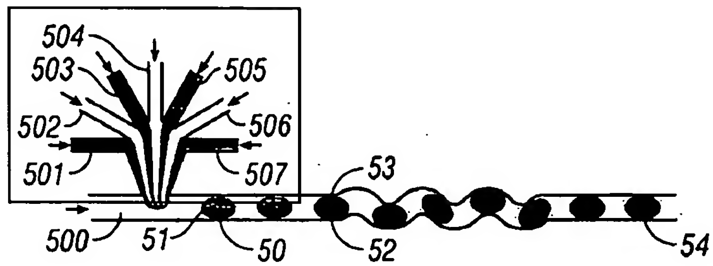

FIG. 5

As shown in the boxed region, the flow of the plug-fluids prior to introduction into the microfluidic channel can initially be laminar. Once these plug-fluids are introduced into the microfluidic channel and contact the carrier-fluid (which is pushed through the channel by applying pressure), plugs form.

The amount of plug-fluids within each plugs can be changed, for example, by changing the relative flow rates of the plug-fluids. A series of plugs, each containing different reagents, can also be formed by introducing different plug-fluids. After plugs form, mixing of the plug-fluids within the plugs can be controlled, for example, by changing the geometry of the microfluidic channels or changing the flow of the plugs/carrier-fluid through the microchannel. Further, the flow of plugs/carrier-fluid through the channel can be slowed or stopped to allow the plugs to incubate. The claimed methods allow a high degree of control over protein and precipitant concentrations as well as a high degree of control over the mixing of these reagents.

The above described methods for forming and mixing an array of different plugs can be used to screen for the optimal crystallization conditions. Once these are determined, the flow rates of the plug-fluids can be maintained at the optimal conditions to form a series of substantially identical plugs (to form larger amounts of crystals).

2. Claim Objection

Claim 6 has been objected to as being of improper dependent form for failing to further limit the subject matter of a previous claim. Claim 6 has been amended to further recite "where the carrier-fluid is permeable to water." Support for amended claim 6 can be found in Applicants' specification, for example, in page 89, lines 12-23. Accordingly, the Applicants respectfully submit that the objection to claim 6 has been overcome and should be withdrawn.

3. Claim Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-7, 9-15, 17, 19, 21-34, 36, 38-44, 49-62, and 75-77 have been rejected under 35 U.S.C. § 112, first paragraph. The Applicants respectfully traverse these rejections based on the following remarks.

Independent claims 1 and 3 have been amended to recite that each plug-fluid is substantially immiscible with the first carrier-fluid. Independent claim 3 has also been amended to read "where the crystallization target forms a crystal in at least one of the at least one or more plugs of the first plug type."

Claims 27-30 have been amended to replace "osmotic pressure" with "osmolarity", which can be regulated, for example, by adjusting the flow rates of the fluids. Support for amended claim 27-30 can be found in the Applicants' specification, for example, in page 90, first full paragraph.

Claims 31-33 have been canceled.

Accordingly, the Applicants respectfully submit that the rejections against claims 1-7, 9-15, 17, 19, 21-34, 36, 38-44, 49-62, and 75-77 have been overcome and should be withdrawn.

4. Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1-7, 9-15, 17, 19, 21-34, 36, 38-44, 49-62, and 75-77 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly

point out and distinctly claim the subject matter which applicant regards as the invention. The Applicants respectfully traverse these rejections based on the following remarks.

Regarding independent claim 1, the term "plug-forming region" is defined in the specification in page 15, lines 22-26. Accordingly, the Applicants respectfully submit that the recitation of "a plug-forming region of the first channel" of claim 1 is clear. Further, independent claim 1 has also been amended to recite "a plurality of plugs forms in the first channel in the first carrier-fluid after the plug-fluids contact the first carrier-fluid." Support for amended independent claim 1 can be found in the Applicants' specification, for example, in Figs. 49, 53, and 54.

Regarding claim 2, the Examiner has asserted that the recitation of the additional plug-fluids is not apparent because "the crystallization already took place" (Office Action, page 4). As discussed in the response to the non-final office action filed January 9, 2008, the plug fluids from the second channel as recited in claim 2 can, for example, merge with the plugs from the first channel to change either the condition of crystal growth (merging occurs after crystallization) or the condition of crystallization (merging occurs before crystallization) (see Figs. 12 and 19, page 41, first paragraph, and the paragraph on pages 86-87). Accordingly, the Applicants respectfully submit that the recitation of the additional plug-fluids of claim 2 is clear.

Regarding independent claim 3, first of all, crystallization prevention is not a recited limitation. The first plug-forming region, the second plug-forming region within the first channel, or the second plug-forming region within a second channel can be plug-forming regions located either in the first channel or in the second channel.

Claim 6 has been previously amended to read "solvent transfer." The Applicants respectfully submit that the antecedent basis is proper.

Claim 10 has been canceled. Claim 9 has been amended to recite "where the one or more second plug-fluids is introduced into at least one of the first plug-forming region and the second-plug-forming region."

Regarding claim 13, the second carrier-fluid can be introduced into either one of the three plug-forming regions. The second carrier-fluid can separate plugs, for example, by forming distinct laminar streams (see, for example, page 26, lines 28-34).

Regarding claims 15 and 24, "substrate" has been replaced with "channels." Claim 23 has been amended to read "where at least one of the channels has a turn."

Regarding claim 24, see page 90, lines 3-20 for an example of the correlation between the first and second components.

Claim 25 has been amended to recite "an indexing marker." Support for amended claim 25 can be found in the Applicants' specification, for example, in page 90, lines 3-20.

Regarding claims 27-30, see discussion in section 2 above.

Regarding claims 40-41, "permeable" is commonly understood to mean "capable of being permeated: penetrable." Merriam-Webster's Collegiate Dictionary (11th ed., can be used in connection with either a solid or a liquid or a gas.

In view of the above amendments and remarks, the Applicants respectfully submit that claims 1-7, 9-15, 17, 19, 21-34, 36, 38-44, 49-62, and 75-77 particularly point out and distinctly claim the subject matter which applicant regards as the invention. Accordingly, the rejections against claims 1-7, 9-15, 17, 19, 21-34, 36, 38-44, 49-62, and 75-77 have been overcome and should be withdrawn.

5. Claim Rejections under 35 U.S.C. § 103

Claims 1-7, 9, 11-15, 17, 19, 21-22, 24-26, 36, 38-44, 51-62, and 75-77 have been rejected under 35 U.S.C. § 103(a) over Weigl et al. (U.S. Pat. No. 6,409,832) in view of Chayen (J. Cryst. Growth, 1999). Claim 23 has been rejected under 35 U.S.C. § 103(a) over Weigl in view of Chayen, and further in view of Bardell et al. (U.S. Pat. Pub. No. 2001/0048900 A1). Claims 49 and 50 have been rejected under 35 U.S.C. § 103(a) over Weigl in view of Chayen, and further in view of Pantoliano et al. (U.S. Pat. No. 6,569,631). The Applicants respectfully traverse these rejections based on the following remarks.

Weigl discloses a microfluidic system where the fluids either form a laminar flow (see Fig. 1; and column 11, line 54 to column 12, line 8) or form a homogeneous solution (see Fig. 2; and column 12, lines 9-19). When a laminar flow is formed, the two fluids do not mix other than by mutual self-diffusion (see column 12, lines 52-54). When a homogeneous solution is formed, the homogeneous solution is transported to the entire microfluidic channel (39) for crystallization (see column 14, lines 21-24; and Fig. 8). As noted by the Examiner, <u>Weigl does not disclose crystallization in plugs</u> formed by the plug-fluids (Office Action, page 7).

Both the laminar flow and the homogenous solution designs are essential for the crystallization methods disclosed by Weigl ("This action [laminar flow] establishes a concentration gradient in device 10, which allows for a very well defined crystallization. Solvent molecules from one stream can diffuse into a parallel stream containing a different solvent and particles. The change in solvent properties along diffusion interface zones 16, 18 can then induce crystallization or precipitation." See column 11, line 64 to column 12, line 3; column 15, lines 36-43. "The protein sample and the reagent are mixed at a certain ratio, and then flow into crystallization channel 15, where a homogeneously mixed solution 22 is slowed or stopped. Crystallization will then occur inside channel 15." See column 12, lines 14-18.) The Applicants thus respectfully submit that the Examiner's proposed modification to Weigl by introducing plugs to the Weigl design would prevent the formation of either a laminar flow or a homogeneous solution, thus defeat the purpose of the Weigl disclosure.

Also, Weigl teaches a high density screening crystallization cartridge 50 (see Fig. 11 and column 14, line 59 to column 15, line 19). The screening is carried out by forming a series of microfluidic channels connected to their respective, separate solution reservoir (see Fig. 11). Weigl does not teach or suggest adjusting the screening conditions by varying flow rate of the fluids in the same microfluidic channel.

Further, although having conceded that Weigl does not disclose crystallization in plugs formed by the plug-fluids (Office Action, page 7), the Examiner nevertheless attempted to cure this deficiency of Weigl by asserting that Chayen teaches "preferences

of microbatch crystallization in droplets suspended in oil, which are similar to plugs separated by an immiscible carrier fluid in microfluidic channels" (Office Action, page 8; emphasis added). Chayen, however, explicitly teaches that in microbatch experiments, "crystals are grown in 1-2 μL drops of a mixture of protein and crystallising agents" (page 434, right column; page 437, right column to page 438, left column; and Fig. 2). The Applicants respectfully submit that the Examiner fails to establish any reason why droplets suspended in oil used in a microbatch experiment as disclosed by Chayen would be similar to plugs formed by plug-fluids in a microfluidic system.

In view of the above remarks, the Applicants respectfully submit that Weigl in view of Chayen do not teach or suggest all the claim limitations as recited in independent claims 1 and 3. Accordingly, the rejections against independent claims 1 and 3, and thus the rejections against claims 2, 4-7, 9, 11-15, 17, 19, 21-26, 36, 38-44, 49-62, and 75-77, which all depend from independent claims 1 and 3, are improper and should be withdrawn.

6. Conclusion

Based on the above amendments and remarks, the Applicants respectfully submit that the claims are in condition for allowance. The examiner is kindly invited to contact the undersigned agent to expedite allowance.

Respectfully submitted,

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